

Figure 1: Space filling representation of the Fv fragment of the antibody 4-4-20

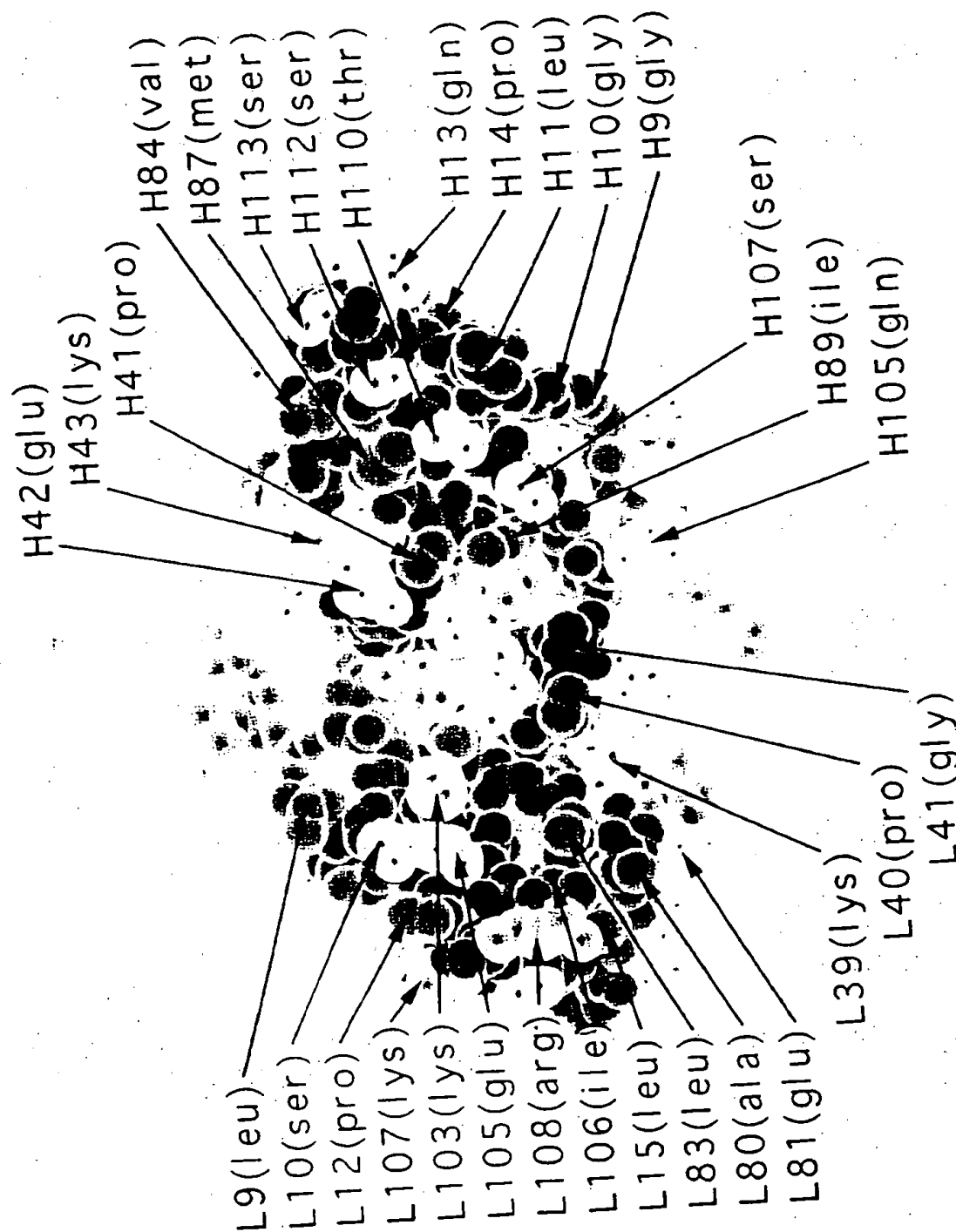


Figure 2a: Variable/constant domain interface residues for VL

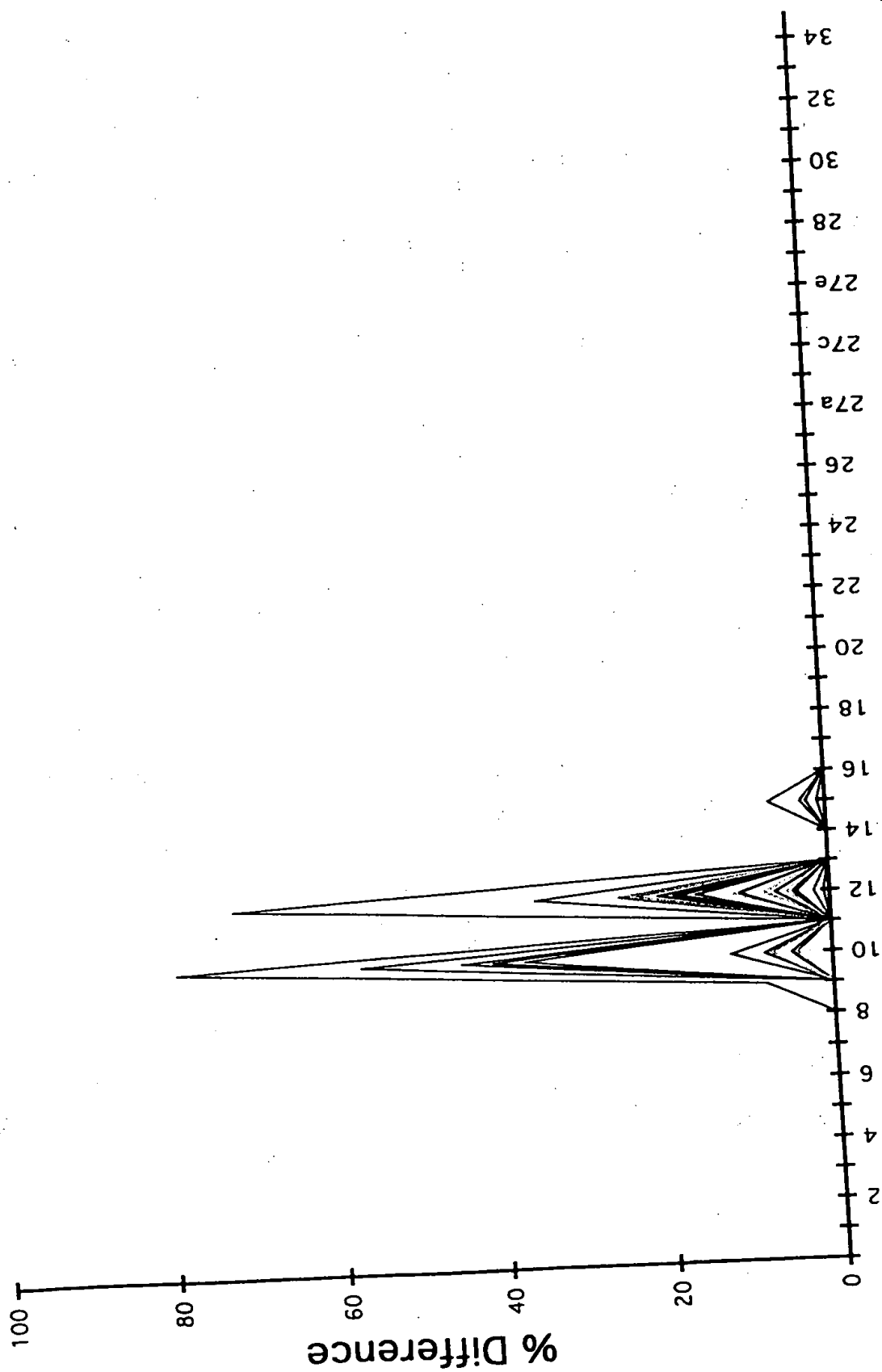


Figure 2a: Variable/constant domain interface residues for VL  
(cont.)

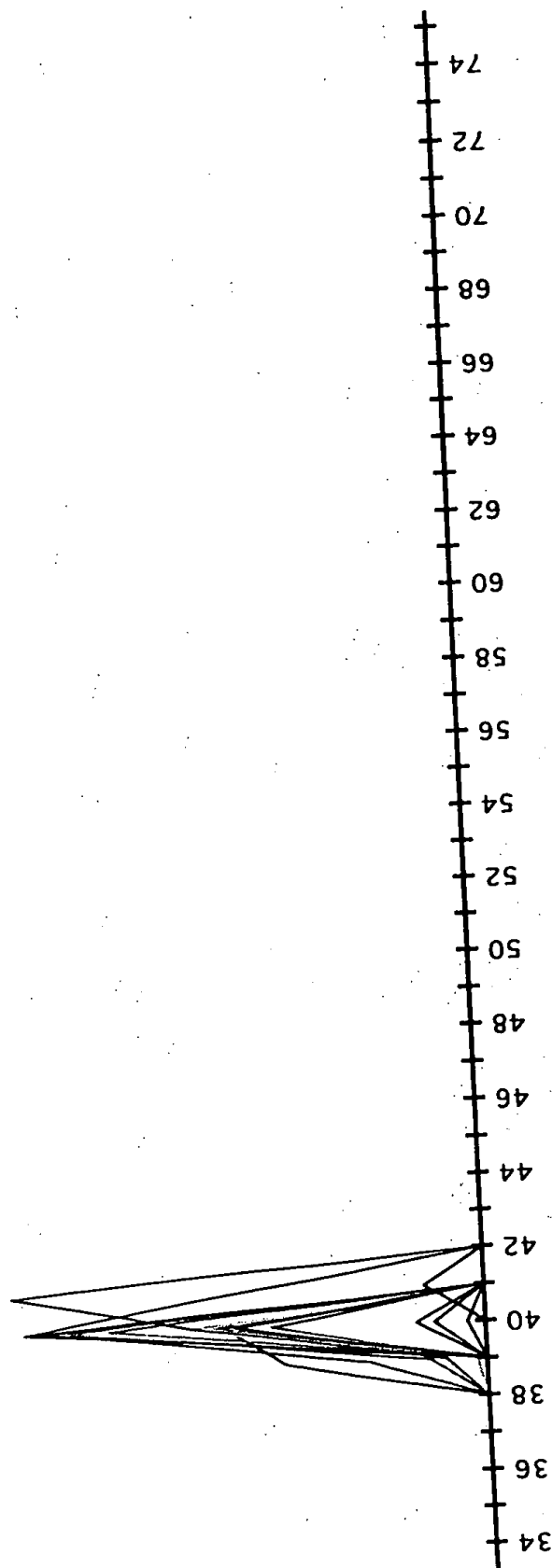


Figure 2a: Variable/constant domain interface residues for VL  
(cont.)

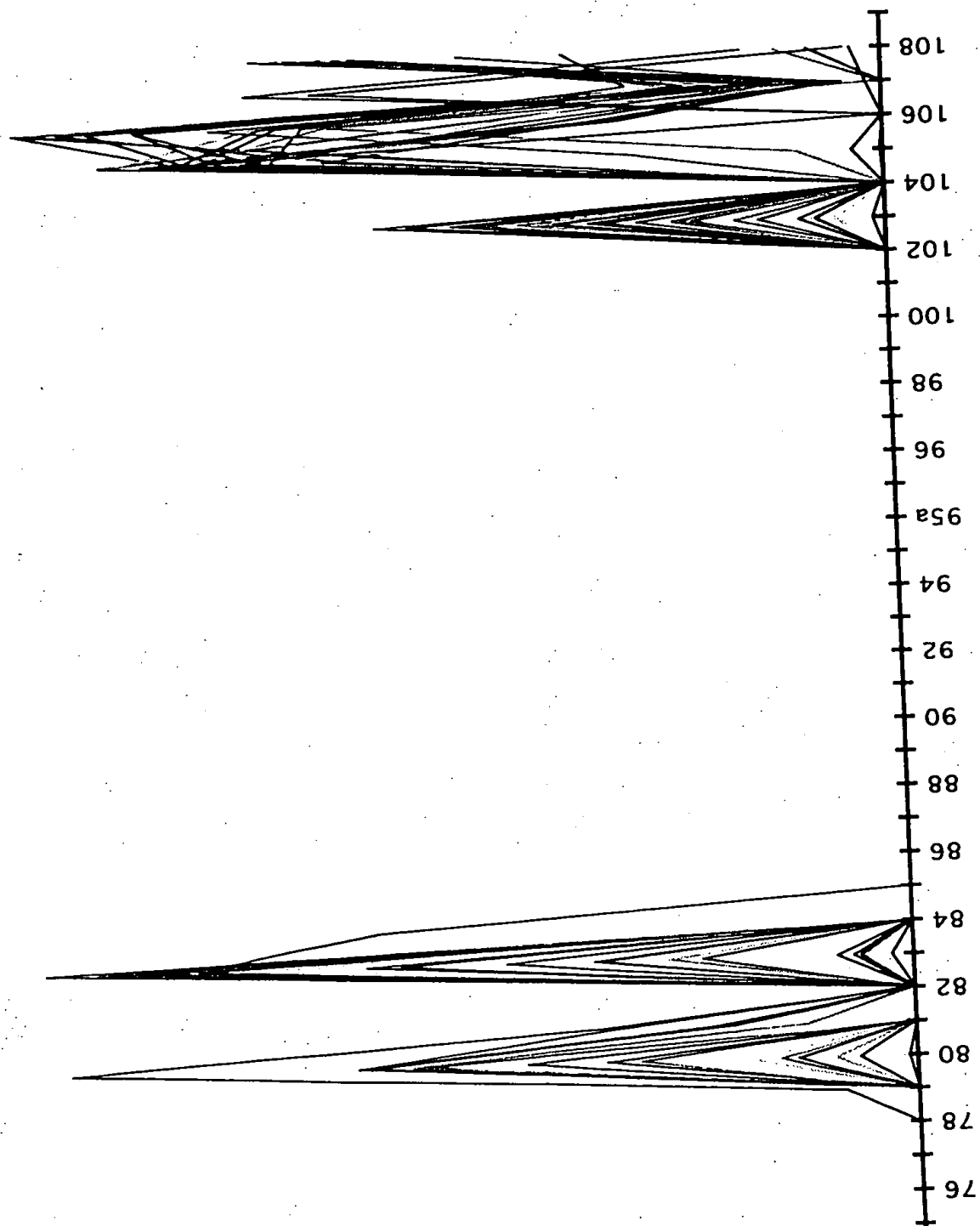


Figure 2a: Variable/constant domain interface residues for VL (cont.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1acy	D	I	V	M	T	Q	S	P	A	S	L	V	V	S	L	G	Q	R	A	T	I	S
1baf	Q	I	V	L	T	Q	S	P	A	I	M	S	S	A	P	G	E	K	V	T	M	T
1bbj	D	I	Q	M	T	Q	S	P	A	S	L	S	S	V	V	G	E	T	V	T	I	T
1cbv	D	V	V	M	T	Q	T	P	L	S	L	P	S	V	L	G	D	Q	A	S	I	S
1dfb	D	I	Q	M	T	Q	S	P	S	T	L	S	S	A	V	G	D	R	V	T	I	T
1fdl	D	I	Q	M	T	Q	S	P	A	S	L	S	S	A	V	G	E	T	V	T	I	T
1fig	E	N	V	L	T	Q	S	P	A	I	M	S	S	A	P	G	E	K	V	T	M	A
1frg	D	I	V	M	T	Q	S	P	S	S	L	T	S	V	A	G	E	K	V	T	M	S
1fvd	D	I	Q	M	T	Q	S	P	S	S	L	S	S	A	V	G	D	R	V	T	I	T
1ggb	D	I	V	L	T	Q	S	P	G	S	L	A	S	V	L	G	Q	R	A	T	I	S
1gig	Q	A	V	V	T	Q	E	S	S	A	L	T	T	T	P	G	E	T	V	T	L	T
1hin	D	I	V	M	T	Q	S	P	S	S	L	T	S	V	A	G	E	K	V	T	M	S
1igi	D	V	V	M	T	Q	T	P	L	S	L	P	S	T	L	G	D	Q	A	S	I	S
1ind	A	V	V	T	Q	E	S	S	S	A	L	T	T	T	P	G	E	T	V	T	L	T
1jel	D	V	L	M	T	Q	T	P	L	S	L	P	S	V	L	G	D	Q	A	S	I	S
1mam	D	I	Q	M	T	Q	T	T	S	S	L	S	S	A	L	G	D	R	V	T	I	S
1mco	P	S	A	L	T	Q	P	S	P	S	A	S	S	G	S	G	Q	S	V	T	I	S
1nca	D	I	V	M	T	Q	S	P	K	F	M	S	S	T	V	G	D	R	V	T	I	T
2cgr	E	L	V	M	T	Q	S	P	L	S	L	P	S	V	L	G	D	Q	A	S	I	S
2dbl	D	V	V	M	T	Q	I	P	L	S	L	P	S	V	L	G	D	Q	A	S	I	S
2f19	D	I	Q	M	T	Q	T	T	S	S	L	S	S	A	L	G	D	R	V	T	I	S
2fb4	Q	S	V	L	T	Q	P	S	P	S	A	S	S	G	T	G	Q	R	V	T	I	S
2fbj	E	I	V	L	T	Q	S	P	A	I	T	A	S	A	P	G	Q	K	V	T	I	T
2hfl	D	I	V	L	T	Q	S	P	A	I	M	S	S	V	L	G	E	K	V	T	M	T
2igf	D	V	L	M	T	Q	T	P	L	S	L	P	S	V	L	G	D	Q	A	S	I	S
2mcp	D	I	V	M	T	Q	S	P	S	S	L	S	S	V	A	G	E	R	V	T	M	S
3hfm	D	I	V	L	T	Q	S	P	A	T	L	S	S	V	P	G	N	S	V	S	L	S
4fab	D	V	V	M	T	Q	T	P	L	S	L	P	S	V	L	G	D	Q	A	S	I	S
6fab	D	I	Q	M	T	Q	I	P	S	S	L	S	S	A	L	G	D	R	V	S	I	S
8fab	E	L	T	Q	P	S	P	P	P	S	V	S	S	V	P	G	Q	T	A	R	I	T

Figure 2a: Variable/constant domain interface residues for VL

23	24	25	26	27	27a	27b	27c	27d	27e	27f	28	29	30	31	32	33	34	35	36	37	38	39	O
C	R	A	S	E	S	V	D			S	Y	G	K	S	F	M	H	W	Y	Q	Q	K	■
C	S	A	S	S								S	V	Y	Y	M	Y	W	Y	Q	Q	K	
C	R	A	S	E							N	I	Y	S	N	L	A	W	Y	Q	Q	K	
C	R	S	S	Q	S	L	V		H	S	N	G	N	T	Y	L	H	W	Y	L	Q	K	
C	R	A	S	Q							S	I	S	R	W	L	A	W	Y	Q	Q	K	
C	R	A	S	Q							N	I	H	N	Y	L	A	W	Y	Q	Q	K	
C	R	A	S	G							V	S	S	T	Y	L	H	W	Y	Q	Q	K	
C	R	A	S	S	S						K	R	K	N	F	L	T	W	Y	H	Q	K	
C	K	S	S	Q	S	L	F	N	S	G	D	V	N	T	A	V	A	W	Y	Q	Q	K	
C	R	A	S	Q							D	D	G	N	S	F	L	H	W	Y	Q	K	
C	R	A	S	E	S	V	D				V	T	T	S	N	Y	A	N	W	Y	Q	E	
C	R	S	S	T	G	A							K	N	Y	L	T	W	Y	Q	Q	K	
C	T	S	S	Q	S	L	F	N	S	H	S	N	G	N	T	Y	L	N	W	Y	L	Q	K
C	R	S	S	Q	S	L	V				V	T	T	S	N	Y	A	N	W	Y	Q	E	K
C	R	S	S	T	G	A					H	G	N	G	N	T	Y	L	E	W	Y	L	K
C	R	S	S	Q	S	I	V						I	Y	N	Y	L	N	W	Y	Q	Q	K
C	R	A	S	Q									G	Y	N	Y	V	S	W	Y	Q	H	
C	T	G	T	S	S	D					V	G	G	Y	N	Y	V	S	W	Y	Q	K	
C	K	A	S	Q							H	S	N	G	N	T	Y	L	H	W	Y	L	K
C	R	P	S	Q	S	L	V				H	S	N	G	N	T	Y	L	H	W	Y	L	K
C	R	S	S	Q	S	L	I						I	S	N	Y	L	N	W	Y	Q	K	
C	R	A	S	Q							N	I	G	S	S	T	V	N	W	Y	Q	L	
C	S	G	T	S	S								S	V	S	S	L	N	W	Y	Q	K	●
C	S	A	S	S									S	V	N	Y	M	Y	W	Y	Q	K	
C	S	A	S	S																		K	
C	R	S	N	Q	T	I	L			L	S	D	G	D	T	Y	L	E	W	Y	L	Q	K
C	K	S	S	Q	S	L	L	N	S	G	N	Q	K	N	F	L	A	W	Y	Q	Q	K	
C	R	A	S	Q							S	I	G	N	N	L	H	W	Y	Q	Q	K	
C	R	S	S	Q	S	L	V				H	S	Q	G	N	T	Y	L	R	W	Y	L	K
C	R	A	S	Q									I	N	N	F	L	N	W	Y	Q	K	
C	S	A	N	A							L	P	N	Q	Y	A	Y	W	Y	Q	Q	K	

Figure 2a: Variable/constant domain interface residues for VL  
(cont.)

Cont.)

40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62		
P	■	Q	P	P	K	V	L	I	Y	I	A	S	N	L	E	S	G	V	P	A	R	F		
P		S	S	P	R	L	L	I	Y	D	T	S	N	L	A	S	G	V	P	V	R	F		
Q	■	K	S	P	Q	L	L	V	Y	A	A	T	N	L	A	D	G	V	P	S	R	F		
P		Q	S	P	K	L	L	I	Y	K	V	S	N	R	F	S	G	V	P	D	R	F		
P	■	K	V	P	K	L	L	I	Y	K	A	S	S	L	E	S	G	V	P	S	R	F		
Q	●	K	S	P	Q	L	L	V	Y	Y	T	T	T	L	A	D	G	V	P	S	R	F		
S	▲	A	S	P	K	L	L	I	Y	S	T	S	N	L	A	S	G	V	P	A	R	F		
P		Q	P	P	K	L	L	I	Y	W	A	S	T	R	E	S	G	V	P	D	R	F		
P	■	K	A	P	K	L	L	I	Y	S	A	S	F	L	E	S	G	V	P	S	R	F		
P	■	Q	P	P	K	L	L	I	Y	R	S	S	N	L	I	S	G	I	P	D	R	F		
P	■	Q	P	P	K	L	L	I	G	G	T	N	N	R	A	P	G	V	P	A	R	F		
P	▲	H	L	F	T	G	L	I	Y	W	A	S	T	R	E	S	G	V	P	D	R	F		
P		Q	P	P	K	V	L	I	Y	K	V	S	N	R	F	S	G	V	P	D	R	F		
A		Q	S	P	K	L	L	I	Y	G	T	N	N	R	A	P	G	V	P	A	R	F		
P	■	H	L	F	T	G	L	I	Y	S	I	S	S	R	F	S	G	V	P	D	R	F		
P	■	Q	S	P	K	L	L	I	Y	Y	T	S	R	L	H	S	G	V	P	S	R	F		
P	■	G	T	V	K	L	L	I	Y	E	V	N	K	R	P	S	G	V	P	D	R	F		
A	■	K	A	P	K	V	L	I	Y	W	A	S	T	R	H	I	G	V	P	D	R	F		
P	■	Q	S	P	K	L	L	I	Y	R	V	S	N	R	F	S	G	V	P	D	R	F		
P	■	Q	S	P	K	L	L	L	Y	K	V	S	N	R	F	F	Y	G	V	P	D	R	F	
P		Q	S	P	K	L	L	L	M	Y	K	V	S	N	R	L	H	S	G	V	P	S	R	F
P		G	T	V	K	L	L	L	I	Y	Y	T	S	R	P	S	G	V	P	D	R	F		
P	●	M	A	P	K	L	L	L	I	Y	R	D	A	M	R	P	S	G	V	P	D	R	F	
P		T	S	P	K	P	W	I	Y	E	I	S	K	L	A	S	G	V	P	A	R	F		
S	▲	T	S	P	K	R	W	I	Y	D	T	S	K	L	A	S	G	V	P	V	R	F		
S	■	Q	S	P	K	L	L	I	Y	K	V	S	N	R	F	S	G	V	P	D	R	F		
P	■	Q	S	P	K	L	L	I	Y	G	A	S	T	R	E	S	G	V	P	D	R	F		
P	▲	Q	P	P	K	L	L	I	Y	K	Y	A	S	Q	S	I	S	G	I	P	S	R	F	
S	▲	E	S	P	R	L	L	I	Y	K	V	S	N	R	F	S	G	V	P	D	R	F		
P		Q	S	P	K	V	L	I	Y	K	V	S	N	R	S	Q	S	G	V	P	S	R	F	
P	■	G	T	I	K	L	L	I	Y	F	T	S	R	S	P	S	G	I	P	Q	R	F		
P		R	A	P	V	M	V	I	Y	K	D	T	Q	R	P	S	G	I	P	Q	R	F		
P	G																							

Figure 2a: Variable/constant domain interface residues for VL  
(cont.)

63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83			
S	G	S	G	S	R	T	D	F	T	L	T	I	D	P	V	E	A	▼	D	■	D	A	◆
S	G	S	G	S	G	T	S	Y	S	L	T	I	S	R	M	E	A		E		D	A	●
S	G	S	G	S	G	T	Q	Y	S	L	K	I	N	S	L	Q	S	▼	E	■	D	F	◆
S	G	S	G	S	G	T	D	F	T	L	K	I	S	R	V	E	A		E		D	L	●
S	G	S	G	S	G	T	E	F	T	L	T	I	S	S	L	Q	P		D		D	F	
S	G	S	G	S	G	T	Q	Y	S	L	K	I	N	S	L	Q	P		E		D	F	
S	G	S	G	S	G	T	S	Y	S	L	T	I	S	S	V	E	●	◆	E	■	D	A	◆
S	G	S	G	S	G	T	D	F	T	L	T	I	T	S	V	Q	A		E		D	L	●
S	G	S	R	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	●	E		D	F	▼
S	G	S	S	S	R	T	D	F	T	L	T	I	N	P	V	E	A		D		D	V	■
S	G	S	S	S	L	I	G	D	K	A	L	T	I	T	G	A	T	■	E		D	E	▲
T	G	S	G	S	G	T	D	F	T	L	T	I	S	S	V	Q	A		E		D	L	●
S	G	S	G	S	G	T	D	F	T	L	K	I	S	R	V	E	A		E		D	L	
S	G	S	L	I	G	D	K	A	A	L	T	I	T	G	A	Q	T		E		D	E	▼
S	G	S	G	S	G	T	D	K	A	L	T	I	S	R	V	Q	A	●	E		D	L	▼
S	G	S	G	S	G	T	D	F	T	L	K	I	S	N	L	N	Q	Q	E		D	M	◆
S	G	S	G	S	G	T	D	Y	S	L	T	I	S	G	L	Q	A		E		D	E	
S	G	S	K	S	G	T	D	Y	T	L	T	I	S	S	V	Q	A	■	E	■	D	L	◆
A	G	S	G	S	G	T	D	Y	T	L	T	I	S	R	V	E	A	▲	E		D	L	◆
S	G	S	G	S	G	T	A	F	T	L	K	I	S	R	V	E	A		E		D	L	
S	G	S	G	S	G	T	D	F	T	L	K	I	S	R	V	E	A		E		D	I	
S	G	S	G	S	G	T	D	Y	S	L	T	I	S	N	L	E	H	●	E		D	E	■
S	G	S	S	S	G	T	D	Y	S	L	A	I	G	G	L	Q	S	●	E	●	D	E	◆
S	G	S	S	S	K	S	A	S	L	T	I	N	T	M	E	E	A	▲	E		D	A	◆
S	G	S	G	S	G	T	S	Y	S	L	T	I	S	S	M	E	T		E		D	A	■
S	G	S	G	S	G	T	S	Y	S	L	T	I	S	S	V	E	A	■	E		D	L	◆
S	G	S	G	S	G	T	D	F	T	L	K	I	S	R	V	E	A		E		D	L	◆
S	G	S	S	S	G	T	D	F	T	L	T	I	S	S	V	Q	A		E	■	D	L	◆
T	G	S	S	S	G	T	D	F	T	L	T	I	S	S	V	E	T	●	E		D	F	◆
S	G	S	S	S	G	T	D	F	T	L	S	I	N	S	V	E	A		E		D	L	▲
S	G	S	S	S	G	T	D	F	T	L	K	I	S	R	V	E	Q	▲	E		D	I	■
S	G	S	G	S	G	T	D	Y	S	L	T	I	S	N	L	E	Q		E		D	E	■
S	S	S	T	S	S	G	T	T	V	T	L	T	I	S	G	V	A		E		D	E	■



Figure 2a: Variable/constant domain interface residues for VL  
(cont.)

84	85	86	87	88	89	90	91	92	93	94	95	95a	95b	96	97	98	99	100	101	102	103
A	T	Y	Y	C	Q	Q	Q	N	N		E	D	P	P	T	F	G	A	G	T	K
A	T	Y	Y	C	Q	Q	Q	W	S	S	Y	P	P	I	T	F	G	V	G	T	K
G	S	Y	Y	C	Q	H	F	W			G	T	P	Y	T	F	G	G	G	T	R
G	V	Y	F	C	S	Q	S	T			H	V	P	L	T	F	G	A	G	T	K
A	T	Y	Y	C	Q	Q	Q	Y				N	S	Y	S	F	G	P	G	T	K
G	S	Y	Y	C	Q	Q	H	F	W		S	T	P	R	T	F	G	G	G	T	K
A	T	Y	Y	C	Q	Q	Y	S			G	Y	P	L	T	F	G	A	G	T	K
A	I	Y	Y	C	Q	N	D	Y	S		S	H	P	L	T	F	G	A	G	T	K
A	T	Y	Y	C	Q	Q	H	Y			T	T	P	P	T	F	G	Q	G	T	K
A	T	Y	Y	C	Q	Q	S	N			E	D	P	L	T	F	G	A	G	T	K
A	T	Y	Y	C	Q	Q	L	W	Y		S	N	H	W	V	F	G	G	G	T	K
A	V	Y	Y	C	Q	N	D	Y			S	N	P	L	T	F	G	G	G	T	K
G	I	Y	F	C	S	Q	T	T			H	V	P	P	T	F	G	G	G	T	K
A	R	Y	F	C	A	L	W	Y			S	N	L	W	V	F	G	G	G	T	K
G	V	Y	Y	C	F	Q	Q	G	S		H	V	P	Y	T	F	G	G	G	T	K
A	T	Y	I	C	Q	Q	G	N			T	L	P	F	T	F	G	S	G	T	K
A	D	Y	Y	C	S	S	Y	E			S	D	N	F	V	F	G	T	G	T	K
A	L	Y	Y	C	Q	Q	H	Y			S	P	P	W	T	F	G	G	G	T	K
G	V	Y	F	C	S	Q	Q	G	T		H	V	P	Y	T	F	G	G	G	T	K
G	I	Y	F	C	S	Q	S	S			H	V	P	P	T	F	G	G	G	T	K
A	T	Y	F	C	Q	Q	Q	G	S		T	L	P	P	T	F	G	G	G	T	K
A	D	Y	Y	C	A	A	W	D		V	S	L	N	A	Y	V	F	T	G	T	K
A	I	Y	Y	C	Q	Q	W	T			Y	P	L	I	T	F	G	A	G	T	K
A	E	Y	Y	C	Q	Q	W				G	R	N	P	T	F	G	G	G	T	K
G	V	Y	Y	C	F	Q	G	S			H	V	P	P	T	F	G	G	G	T	K
A	V	Y	Y	C	Q	N	D	H			S	Y	P	L	T	F	G	A	G	T	K
G	M	Y	F	C	Q	Q	S	N			S	W	P	Y	T	F	G	G	G	T	K
G	V	Y	F	C	S	Q	S	T			H	V	P	P	T	F	G	G	G	T	K
A	T	Y	F	C	Q	Q	G	N			A	L	P	P	T	F	G	G	G	T	K
A	D	Y	Y	C	Q	A	W	D			N	S	A	S	I	F	G	G	G	T	K

[illegible]

Figure 2b: Variable/constant domain interface residues for VH

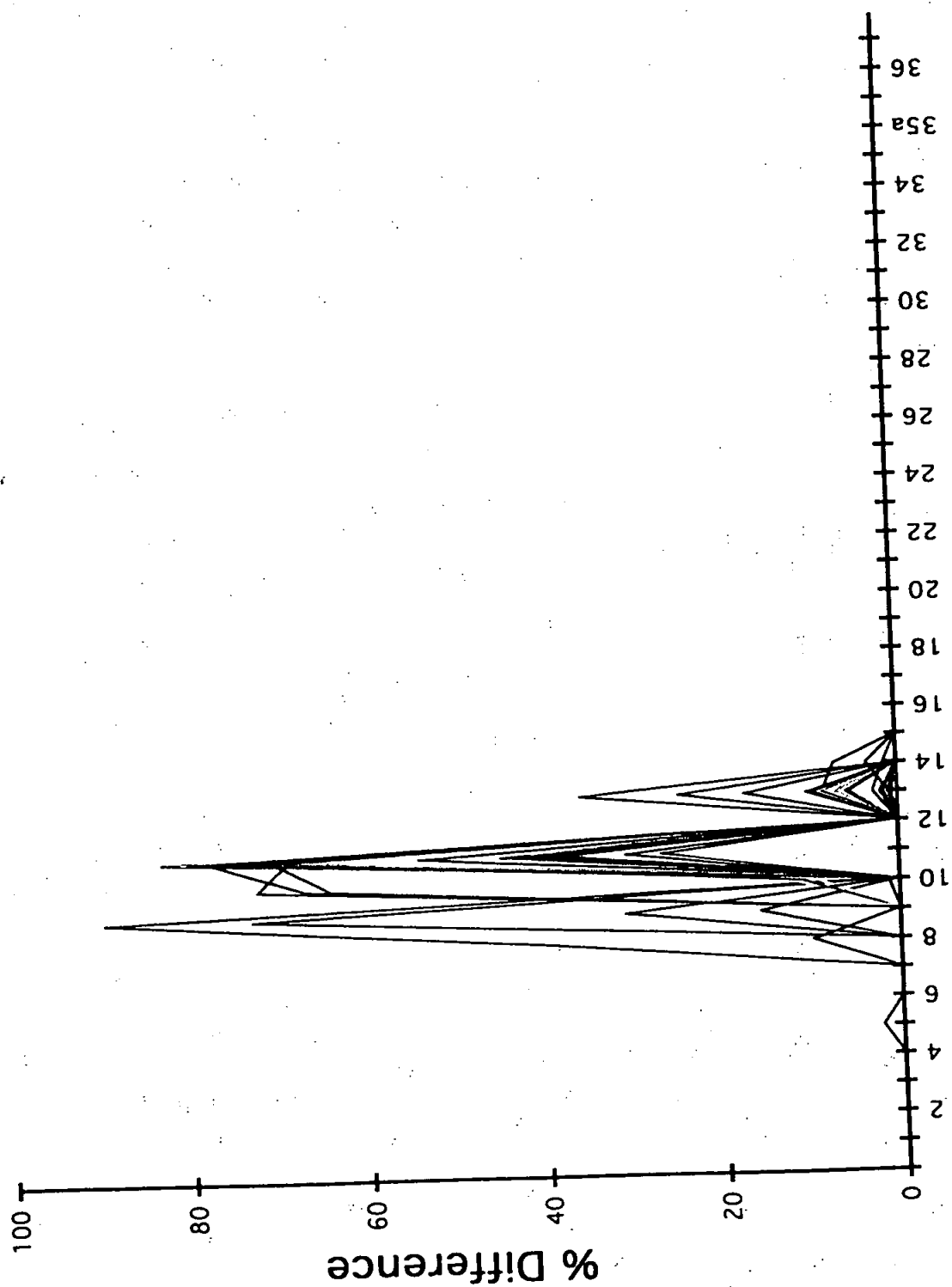


Figure 2b: Variable/constant domain interface residues for VH  
(cont.)

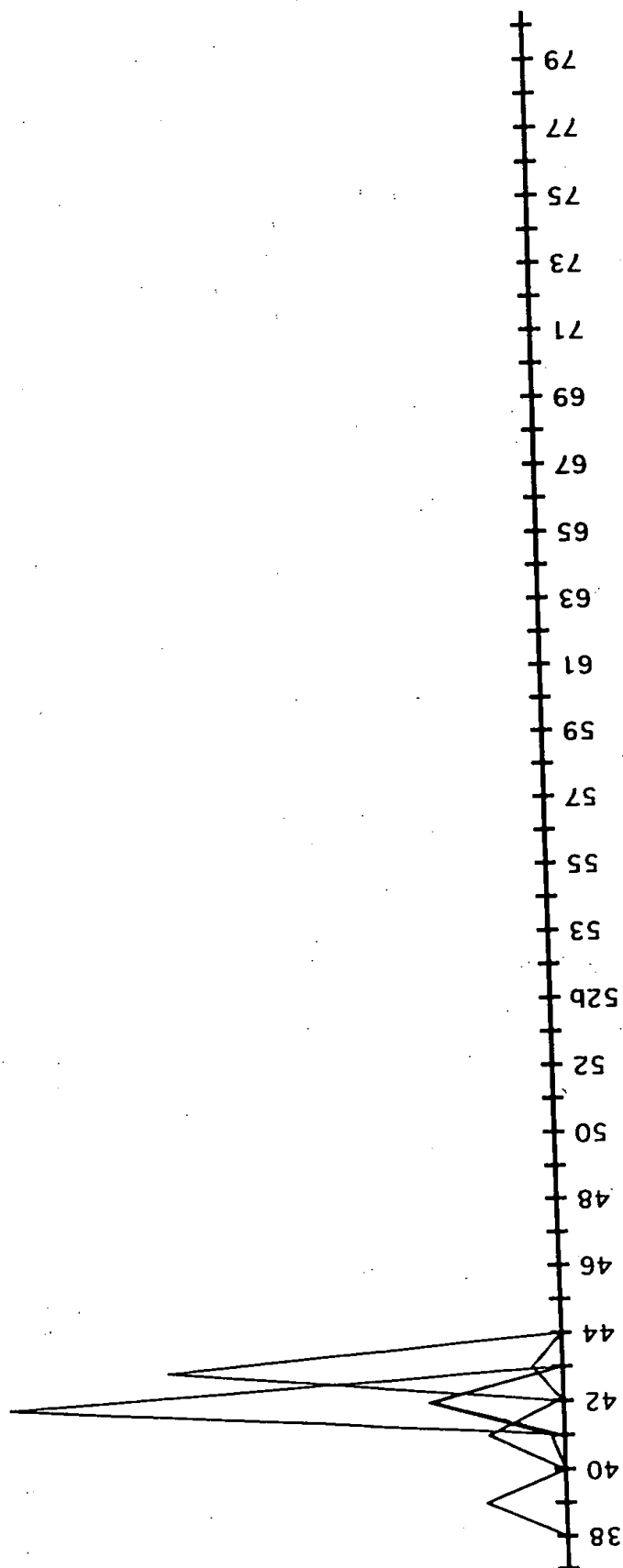


Figure 2b: Variable/constant domain interface residues for VH  
(cont.)

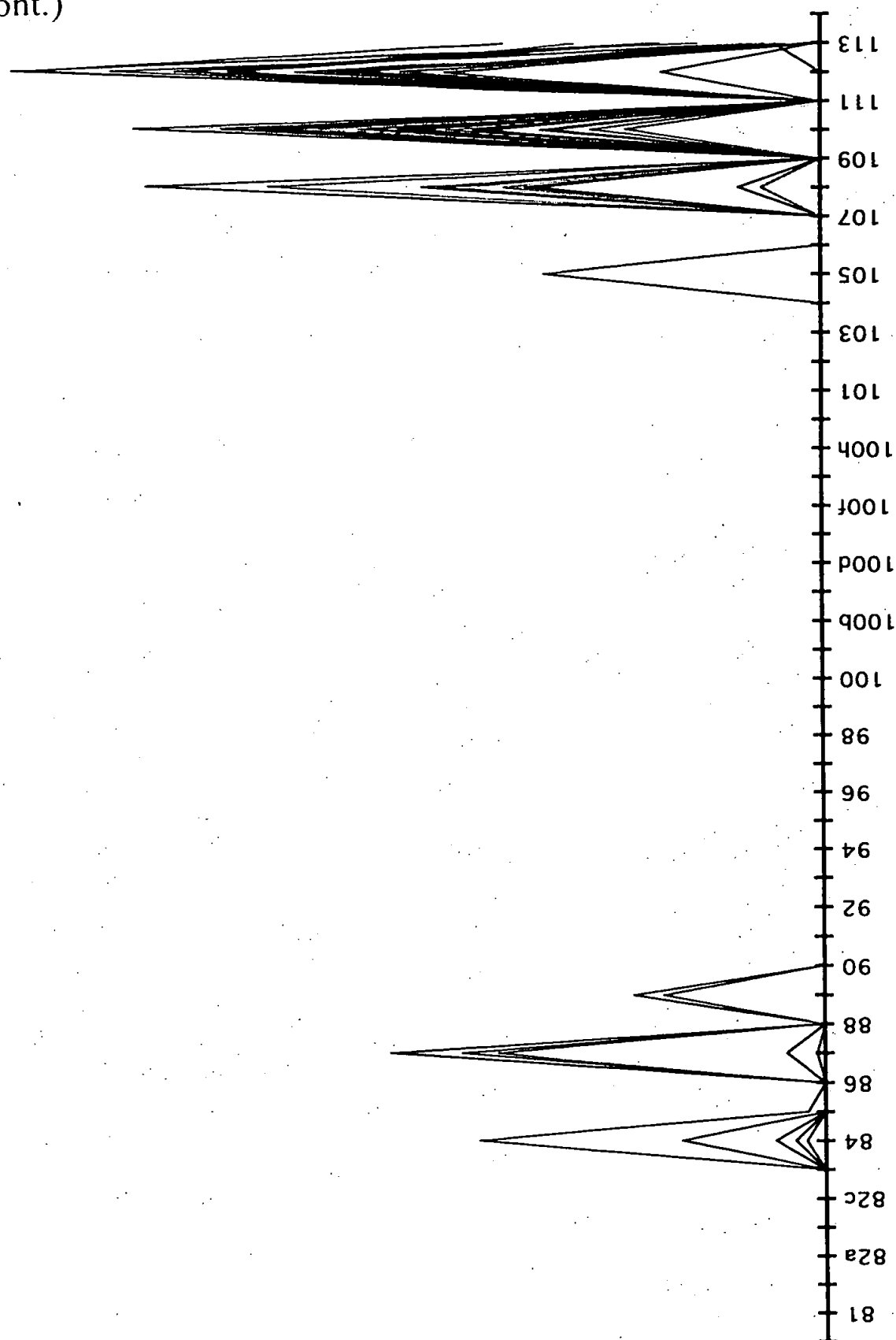


Figure 2b: Variable/constant domain interface residues for VH  
(cont.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1acy	Q	V	K	L	Q	E	S	G	P	A	V	I	K	P	S	Q	S	L	S	L	T	C
1baf	D	V	Q	L	Q	E	S	G	P	G	L	V	K	P	S	Q	S	L	S	L	T	C
1bbj		V	Q	L	V	E	S	D	A	E	L	V	K	P	S	A	S	L	S	L	T	C
1cbv	E	V	Q	P	V	E	S	G	G	G	L	V	Q	P	S	G	S	L	S	L	T	C
1dfb	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	S	G	S	L	S	L	T	C
1fdl	Q	V	Q	L	K	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
1fig	D	V	Q	L	V	E	S	G	P	D	L	V	Q	P	S	G	S	L	S	L	T	C
1frg	E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	S	G	S	L	S	L	T	C
1fvd	Q	V	Q	L	K	E	S	G	P	G	L	V	Q	P	S	G	S	L	S	L	T	C
1ggb	Q	V	Q	L	V	E	S	G	P	G	L	V	Q	P	S	G	S	L	S	L	T	C
1gig	E	V	Q	L	V	E	S	G	P	D	L	V	Q	P	S	G	S	L	S	L	T	C
1hin	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
1igi	E	V	Q	L	V	E	S	G	P	D	L	V	Q	P	S	G	S	L	S	L	T	C
1ind	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
1jel	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
1mam	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
1mco	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
1nca	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
2cgr	Q	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
2dbl	Q	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
2f19	Q	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
2fb4	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
2fbj	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
2hfl	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
2igf	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
2mcp	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
3hfm	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
4fab	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
6fab	E	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C
8fab	A	V	Q	L	V	E	S	G	P	E	L	V	Q	P	S	G	S	L	S	L	T	C

Figure 2b: Variable/constant domain interface residues for VH  
(cont.)

23	I	T	K	A	A	T	K	A	A	S	T	A	K	A	K	A	T	K	K	K	K	S	A	K	A	A	S	V	K	I	
24	V	V	A	A	A	V	A	A	A	F	V	A	S	A	G	T	V	A	A	A	A	S	A	A	A	T	V	A	A	A	
25	S	T	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	T	S	S	S	S	S	S	S	S	T	S	S	S	
26	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	
27	F	Y	Y	F	F	F	F	F	F	F	F	F	Y	F	Y	F	D	Y	Y	Y	Y	F	F	Y	F	F	D	F	Y	F	
28	S	S	T	S	T	S	S	T	N	S	L	S	I	T	T	T	S	T	T	A	T	I	D	T	T	T	S	T	T	T	
29	I	I	F	F	F	L	L	F	I	L	L	F	F	L	F	F	I	F	F	F	F	F	F	F	F	F	I	F	F	F	
30	T	T	T	N	N	T	P	S	K	S	I	S	T	S	T	T	N	T	S	T	T	S	S	S	S	S	T	S	T	S	
31	R	T	S																												
32	T	S																													
33	N	D	D	T	D	G	G	S	D	G	S	S	D	G	T	D	L	N	E	N	S	S	K	D	R	D	S	D	S	N	
34	Y	Y	H	N	Y	Y	H	F	T	M	N	Y	F	E	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	C	F	D	Y	N	Y
35	C	A	A	A	A	G	N	G	Y	G	G	G	Y	T	A	Y	Y	G	W	G	G	A	W	W	A	Y	Y	W	G	G	
35a	W	W	I	M	M	V	I	M	I	V	V	M	M	M	M	M	W	M	I	V	V	M	M	I	M	M	W	M	I	M	
35b	H	N	H	N	H	N	N	S	H	S	H	S	N	S	H	S	S	N	E	N	N	Y	S	E	S	E	S	N	N	H	
36	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	
37	I	I	A	V	V	V	I	V	V	I	V	V	V	V	V	V	I	V	V	V	V	V	V	V	V	V	I	V	V	V	
38	R	R	K	R	R	R	V	R	R	R	R	R	R	R	R	K	R	R	K	K	K	K	R	R	K	R	R	R	R	K	R
39	Q	Q	Q	Q	Q	Q	H	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	E	E	Q	Q	Q	Q	Q	Q	Q	K	Q	Q	Q	
40	A	F	K	A	A	P	R	T	A	P	P	T	S	T	S	P	P	A	R	A	R	A	A	R	T	P	F	S	R	A	
41	P	P	P	P	P	P	N	P	P	S	P	P	H	P	H	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
42	G	G	E	G	G	G	G	D	G	G	G	D	G	E	A	G	G	G	G	G	G	G	G	G	E	G	G	E	G	G	
43	K	N	Q	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	H	K	Q	K	K	H	K	K	N	K	Q	K	

Figure 2b: Variable/constant domain interface residues for VH  
(cont.)

(Cont.)	
44	G K G G G G S R G G G R S R S A G G G E G G G R R R G G G
45	L L
46	E E
47	W W
48	M M I V V L I V V L L V I V I L I M I M I V I I V I M V I V
49	G G G A S G G A A A G A G A G G G G A G G A A G A G A
50	R Y Y R G M N T R H V T Y T L F Y W E W Y I E E G A Y Q Y V
51	I M I I I I I I I I I I T I I I I I I I I I I I S V I N I
52	C S S R S W D S Y F W S S L S R Y N L N N W H L S R S R N W
52a	Y Y P S W G P N P W A N P S P N Y T P I P D P P S N Y N P Y
52b	E S G K D D Y G T D G G Y G S K S N G Y G D D G G K S K G N
52c	
53	S N
54	N N S Y G N G S G S G T S T K G S S G K N Y N G
55	G G D Y S G G G G G G G G G G Y G G G G S G G S Y G Y G S
56	S S D A S N G Y Y D N Y V F Y T S E R E Y D T S Y T S E Y R
57	I T I T I T T T T K T T T T T T T T P T P L Q I T T T T T I T
58	Y R K Y G D N Y R R N Y G F S E Y T N T S H N N F E Y Y A Y
59	Y Y Y Y Y Y F Y Y Y Y Y Y Y Y Y G Y Y Y Y Y Y Y Y Y Y Y
60	S N N A A N N Q A N N P N S N S N G R V N A T H P S N S N G
61	P P E D D S P D D P S D Q A G A P E E D E D P E D A P D E D
62	S S K S S A K S S S A S K S E S S E K D K S S R T S S S K S
63	I L F V V L F V V L L V F V F V L F F F F V L F V V L V F V
64	K R K K K K K K K M K K K K K K K K K K K K K K K K K K



ESTO" 0622E260

Figure 2b: Variable/constant domain interface residues for VH  
(cont.)

65	S	S	G	D	G	S	G	G	G	S	S	G	G	G	G	G	S	G	G	G	G	G	D	G	G	G	S	G	G	G	
66	R	R	K	R	R	R	K	R	R	R	R	R	K	R	K	R	R	K	R	K	R	K	K	R	R	R	R	K	R		
67	S	I	A	F	F	L	A	F	F	L	V	F	A	F	A	F	V	F	A	F	T	F	F	A	F	F	I	F	T	F	
68	T	S	T	T	T	S	T	T	K	S	T	T	T	T	T	T	A	T	A	T	T	I	T	I	I	S	T	T	T		
69	I	I	L	I	I	I	L	I	I	I	I	L	I	M	I	I	F	F	F	L	I	I	F	I	V	I	I	L	I		
70	S	T	T	S	S	T	S	S	S	S	T	S	T	S	S	T	S	T	S	T	S	T	S	T	S	T	S	T	S		
71	R	R	A	R	R	K	V	R	A	K	K	R	V	R	V	R	V	L	A	L	V	R	R	A	R	R	R	R	V	R	
72	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	N	E	D	E	D	N	D	D	N	D	D	D	D	D		
73	T	T	K	D	N	N	K	N	T	T	N	N	K	N	K	N	T	T	T	T	R	D	N	T	N	T	T	D	K	N	
74	S	S	S	S	A	S	S	A	S	S	S	A	S	A	S	S	S	S	S	S	S	S	A	S	A	S	S	S	S	S	
75	L	K	S	Q	K	K	S	K	K	N	K	K	S	Q	S	Q	K	A	S	A	S	K	K	S	R	Q	K	K	S	K	
76	N	N	S	N	N	S	S	N	N	N	S	N	S	N	S	S	N	S	N	S	S	N	N	S	N	S	N	S	S	R	
77	K	Q	T	M	S	Q	T	T	T	Q	Q	T	T	N	T	I	Q	T	T	T	T	S	T	T	T	I	Q	S	T	T	
78	F	F	A	L	L	V	L	L	L	V	V	L	A	L	A	L	F	A	A	A	A	L	L	A	L	L	Y	V	A	L	
79	F	F	Y	Y	Y	F	Y	F	Y	F	Y	F	Y	Y	Y	Y	N	Y	Y	Y	F	Y	Y	S	Y	Y	Y	Y	Y		
80	I	L	M	L	L	L	M	L	L	L	L	M	L	M	L	S	L	M	L	M	L	L	M	L	L	L	L	M	M		
81	Q	Q	Q	Q	Q	K	H	E	Q	K	K	Q	E	Q	E	Q	K	Q	Q	E	Q	Q	Q	Q	Q	Q	Q	D	Q	Q	
82	L	L	L	M	M	L	M	M	I	M	M	L	L	L	M	L	I	L	I	L	M	M	L	M	M	L	M	L	M		
82a	I	K	N	N	N	N	T	T	N	T	K	S	R	N	A	N	S	N	S	N	R	D	S	N	S	N	N	N	R	N	
82b	S	S	S	N	S	S	S	S	S	S	S	S	S	S	S	R	T	S	N	S	N	S	S	K	S	S	A	S	N	S	S
82c	V	V	L	L	L	L	L	L	L	V	L	L	L	L	L	V	L	L	L	L	V	L	L	L	V	L	L	L	L		
83	T	T	T	K	R	H	Q	K	R	D	Q	K	T	R	T	R	T	K	T	K	T	R	R	T	R	R	T	R	T	R	
84	N	T	S	T	A	T	S	S	A	T	T	S	S	S	S	A	A	N	S	N	S	P	S	S	S	S	A	T	V	S	T

# DISCOUNT

18/28



Figure 3: Western blots showing the insoluble (i) and soluble (s) fractions of cell extracts

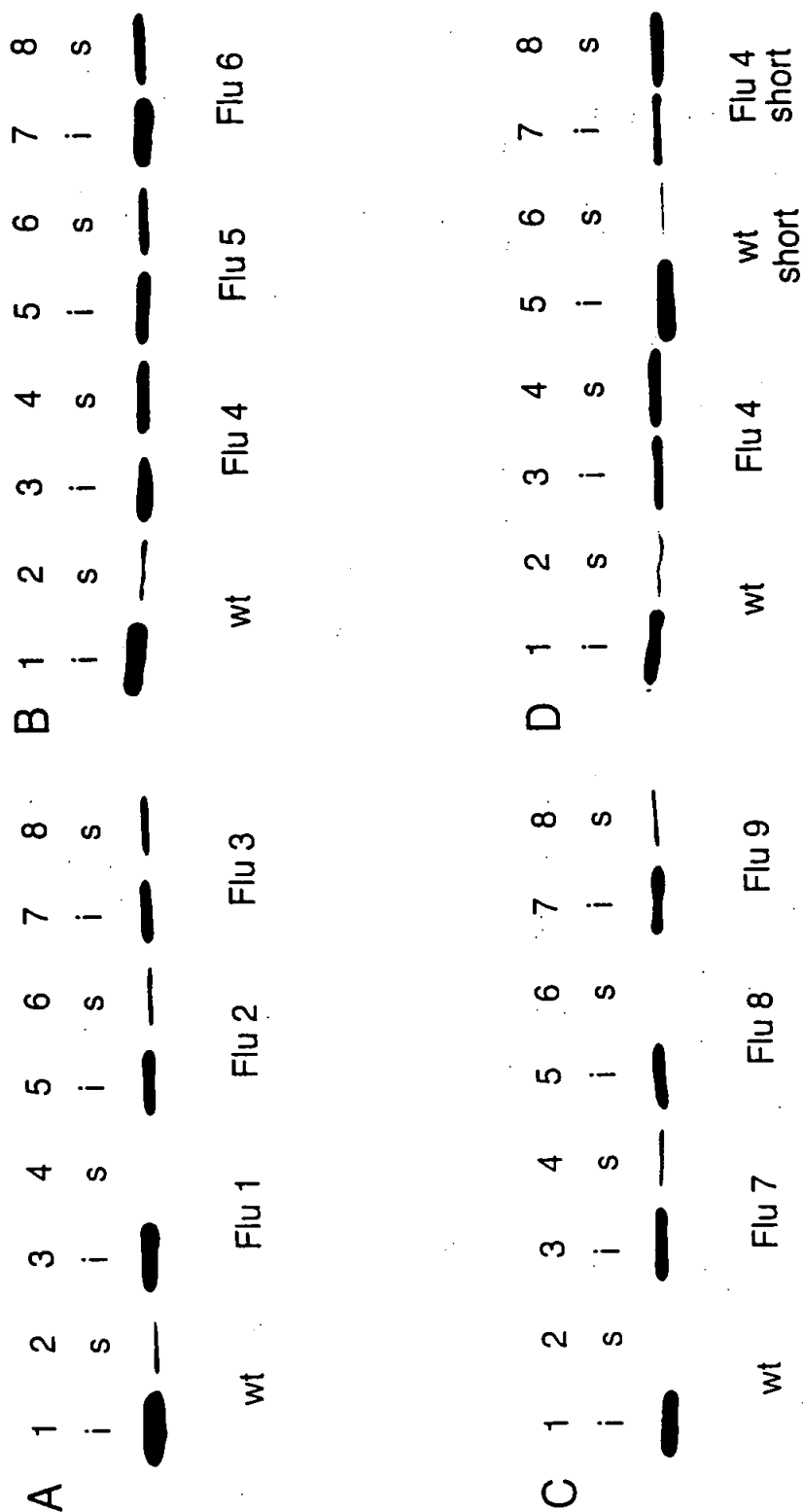


Figure 4: Scatchard plots of fluorescence titration of fluorescein with antibody: a) Titration of wt scFv; b) Titration of Flu4(V84D)

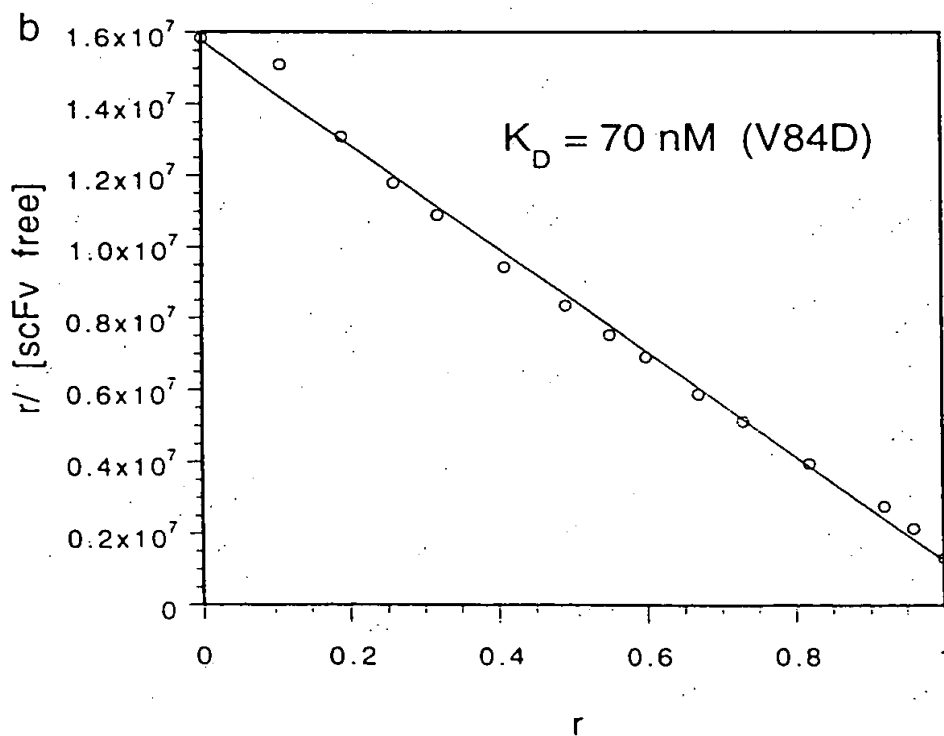
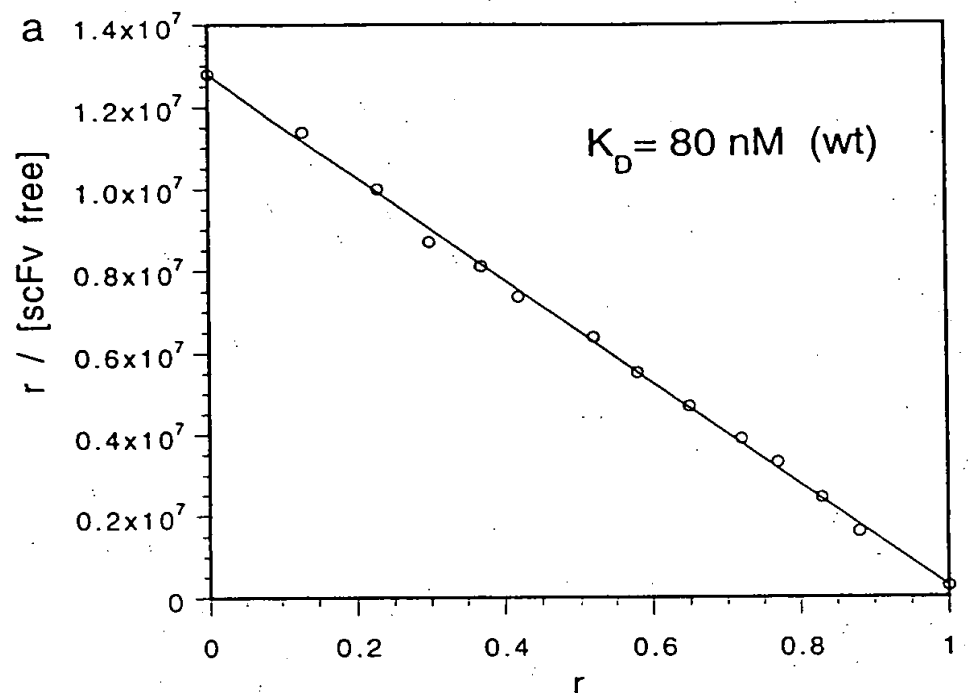


Figure 5: Overlay plot of urea denaturation. (x) wt scFv, (o) Flu4

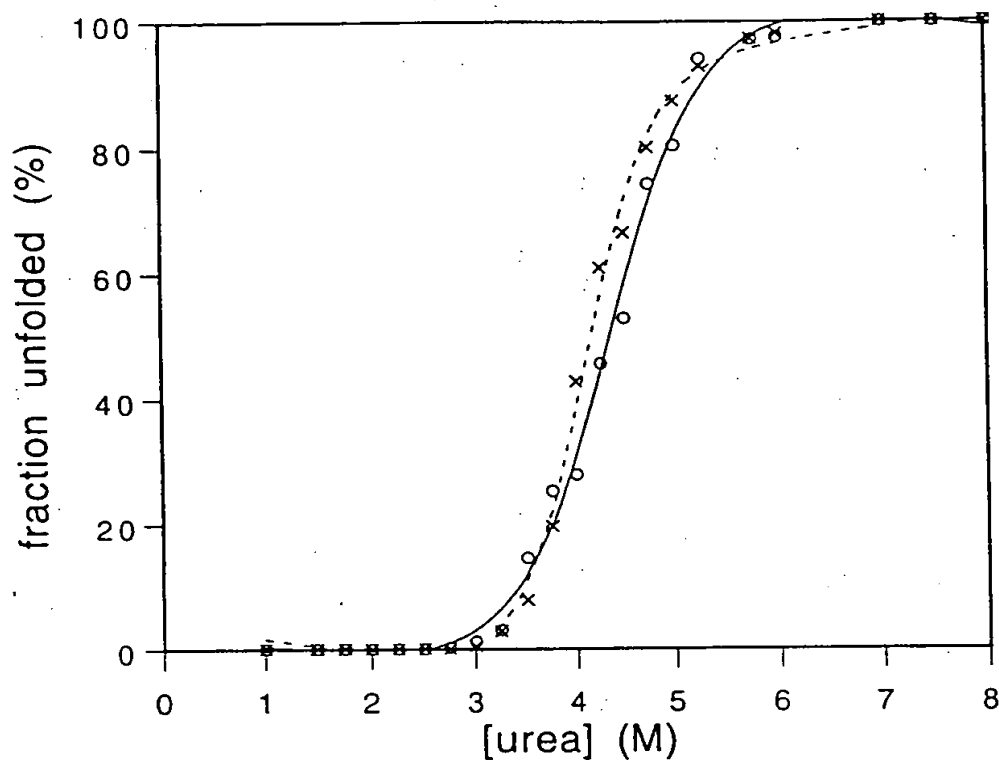


Figure 6: Thermal denaturation time courses at 40°C and 44°C for wt and Flu4 scFv fragments

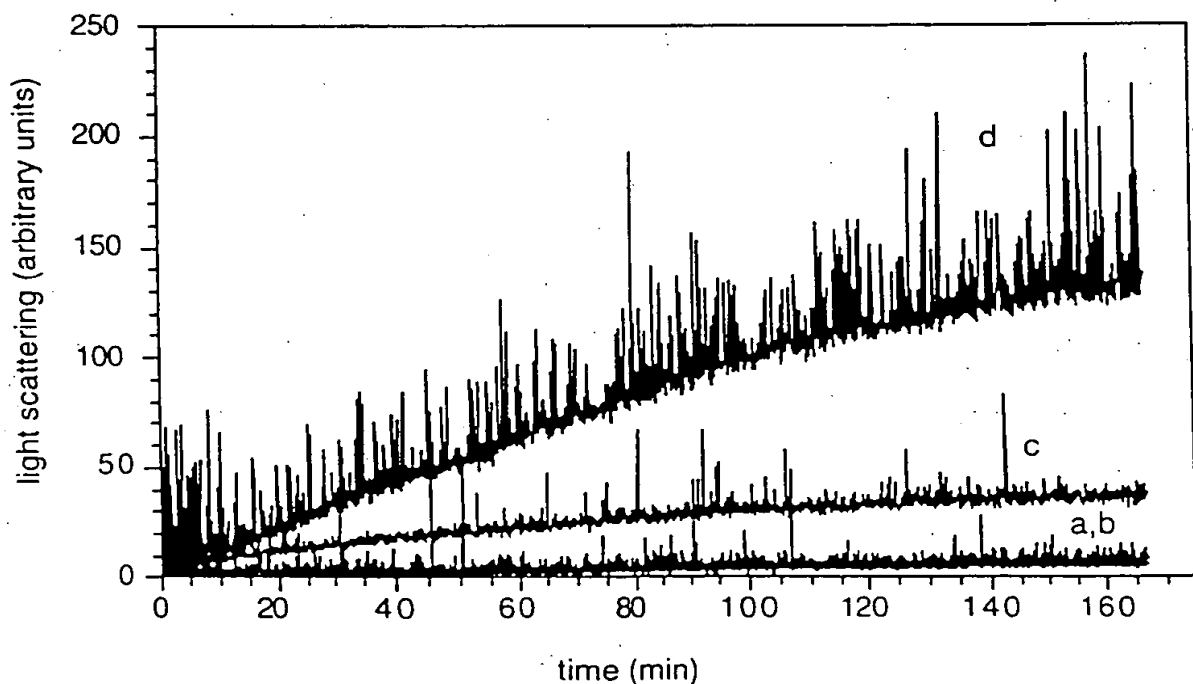


Table 1: Sequence variability of residues contributing to the v/c interface

	L9						L10						L12						L15						L39					
	kappa			lambda			kappa			lambda			kappa			lambda			kappa			lambda			kappa			lambda		
	hu	mu		hu	mu		hu	mu		hu	mu		hu	mu		hu	mu		hu	mu		hu	mu		hu	mu		hu	mu	
	Leu						Ser						Pro						Leu						Lys					
	Ser	Ala	Ser	Ala	Ser	Thr	Thr	Ser	Ser	Ser	Ser	Thr	Ser	Ser	Ser	Pro	Leu	Pro	Pro	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys
Asp	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glu	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lys	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
His	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	0	4	0	0	1	49	6	0	3	0	0	93	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ser	43	24	90	4	48	60	60	84	50	99	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Asn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gly	25	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ala	11	41	7	90	0	0	0	4	23	0	0	0	0	0	0	0	8	1	0	0	0	0	0	0	0	0	0	0	0	0
Cys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	0	2	0	0	0	0	0	11	20	0	0	0	0	0	0	45	28	84	96	0	0	0	0	0	0	0	0	0	0	0
Val	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	41	10	0	0	0	0	0	0	0	0	0	0	0	0	0
Ile	0	0	0	3	0	24	0	0	0	0	0	1	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	11	19	0	0	0	0	0	0	0	0	0	0	0	0	0	11	49	14	4	0	0	0	0	0	0	0	0	0	0	0
Met	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phe	0	1	0	0	0	2	7	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Tyr	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Position  
% exp. (FAB)  
% exp. (ind.)  
% buried v/c

Species  
Seq. 4-4-20  
Consensus  
Distribution:

Table 1: Sequence variability of residues contributing to the v/c interface (cont.)

L40				L41				L80				L81				L83				L103			
kappa	hu	mu	Pro	kappa	hu	mu	Gly	kappa	hu	mu	Ala	kappa	hu	mu	Glu	kappa	hu	mu	Leu	Phe	Glu	Glu	Lys
64	114			57				70				17				42							
82	121			74				74				38				58							
22	3			21				5				46				26							
kappa	hu	mu	Pro	kappa	hu	mu	Gly	kappa	hu	mu	Ala	kappa	hu	mu	Glu	kappa	hu	mu	Leu	Phe	Glu	Glu	Lys
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	19	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
94	77	92	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



Table 1: Sequence variability of residues contributing to the v/c interface (cont.)

[illegible]

Table 1: Sequence variability of residues contributing to the v/c interface (cont.)

Position	H9			H10			H11			H13			H14			H41			H42			H43		
	hu	mu	Gly	hu	mu	Gly	hu	mu	Leu	hu	mu	Gln	hu	mu	Pro	hu	mu	Pro	hu	mu	Glu	hu	mu	Lys
Seq. 4-4-20																								
Consensus																								
Distribution:																								
Asp	0	0	0	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Glu	2	0	0	27	54	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	19	0
Lys	0	0	0	0	0	0	0	0	0	59	54	0	0	0	0	0	0	0	0	0	0	0	0	1
Arg	0	0	0	1	0	0	0	0	0	3	18	0	0	0	0	0	0	0	0	0	0	0	0	0
His	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thr	1	2	1	1	0	0	0	0	0	0	0	0	0	0	1	10	13	1	1	1	0	0	0	0
Ser	1	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Asn	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gln	0	0	0	0	0	0	0	0	1	34	22	0	0	0	0	0	0	0	0	0	0	0	76	0
Gly	42	29	61	38	1	0	0	0	0	0	0	0	0	0	0	59	7	0	0	0	0	1	2	0
Ala	33	31	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cys	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pro	21	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Val	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
Ile	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leu	0	0	0	0	0	0	0	0	60	95	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Met	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phe	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tyr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



Table 2: Mutations introduced in the scFv fragment of the antibody 4-4-20

	L15E (VL)	L11N (VH)	L11D (VH)	V84D (VH)
Flu 1	•			
Flu 2		•		
Flu 3			•	
Flu 4				•
Flu 5		•		•
Flu 6			•	•
Flu 7	•	•		
Flu 8	•		•	
Flu 9	•	•		•
Flu 4 short				•

Table 3:  $K_D$  values of the different scFv mutants determined in fluorescence titration

	Flu wt	Flu 3	Flu 4	Flu 6	Flu wt#
$K_D$ (nM)	$80 \pm 7$	$60 \pm 12$	$70 \pm 10$	$75 \pm 13$	90